10 Metabolism of Insecticides by Microorganisms

F. MATSUMURA and G. M. BOUSH

Department of Entomology
University of Wisconsin
Madison, Wisconsin

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10-1. INTRODUCTION

While many scientists are currently involved in heated debate over the actual or potential injury to ecosystems resulting from continued exposure to pesticides, one thing is apparent: Some pesticides do persist after application, even at recommended rates, for considerable periods of time (*1*).

Among pesticides, insecticides generally seem to pose much more of a hazard to our environment than do either herbicides or fungicides (*2,3*). This is especially true of certain synthetic chlorinated hydrocarbons such as DDT and its analogs, and the cyclodienes aldrin, dieldrin, heptachlor, and endrin. In discussing the biological effects of insecticides, it is extremely important that we be specific, however, since individual members within any group vary manyfold in toxicity. Among the cyclodienes, as is the case with the DDT group, the variation in environmental hazard is great. For example, dieldrin no doubt represents a 100-fold or greater danger to our ecosystem than does endosulfan (a derivative of dieldrin) because of the chemical instability of the latter compound. However, it is equally important to stress the fact that unstable compounds may not always be safe since metabolism of these compounds by different organisms or under different physical or

chemical conditions might yield metabolites considerably more hazardous to the environment than the parent compound.

Most of the persistent organic pesticides, such as those previously mentioned, have been in worldwide usage for about 20 years. No insecticide that has been placed on the market within the past 10–12 years appears to approach the potential environmental threat posed by the above-mentioned older synthetics.

Perhaps it is advisable at this point to consider the chemical and biological characteristics of the chlorinated hydrocarbon insecticides upon which the emphasis of this chapter is focused. Among the chlorinated hydrocarbon insecticides, persistence may not be in the form of the parent compound. In soil and plants aldrin is readily converted to dieldrin and, likewise, heptachlor is converted to the epoxide form. In both cases the molecule is altered to a more stable and at least equally toxic form. However, both DDT and dieldrin themselves are toxic and extremely stable. Until recently, for instance, no degradative metabolites of dieldrin were known (*4*). As might be expected from their persistence, these compounds are of low volatility (i.e., the vapor pressures of DDT and dieldrin are in the ranges of 10^{-7} and 10^{-5} m Hg, respectively) and have extremely low solubility in water [i.e., DDT, 1.2 ppb (*5*), and dieldrin, approximately 0.25 ppm]. In addition, they are extremely apolar. Consequently, the most important property of these chlorinated hydrocarbons is their affinity for fat, which allows them to be retained in plant cuticular waxes, animal fat, and other nonpolar organic matter in the environment. This retention, when passed upward in a food chain, can bring about progressive concentration of the pesticide to a point at which the amount of poison exceeds the tolerance of the organism occupying a certain level of the chain. Differences in the susceptibilities of animal and microbial life to these insecticides are enormous. In some instances, therefore, even a slight concentration is a serious threat; for example, 1 ppm of aldrin in the ration of quail results in 100% adult mortality (*6*).

10-2. TERMINAL RESIDUE CONCEPT

In the past, detection of residues resulting from use of insecticides was in many cases based on extrapolation from total organic chlorine determinations which did not distinguish metabolites from parent compounds. As most of the compounds were noteworthy for their persistence, it was apparently assumed that the molecule remained unaltered after application, or if slowly degraded, always to a less toxic form. Part of this complacency was no doubt attributable to the relatively small quantities of pesticides required per acre as compared with the previously used much less effective inorganics. It was difficult to believe then that one-half, or three-quarters, of a pound of a pesticide broadcast over an acre of soil could be potentially harmful. It later

proved to be a revelation to learn of the transformation in soil of aldrin to dieldrin and heptachlor to heptachlor epoxide. Neither transformed compound could be viewed as a nontoxic metabolite but rather had to be recognized as a more persistent and at least as toxic a product as the original compound. Alteration to a more stable form than that of the parent compound has given rise to the phrase "terminal residue" (7). Actually, such a term is inappropriate since such compounds are probably never the end product of chemical degradation or biological metabolism. Perhaps they should be called "stable toxic intermediates," as this certainly describes them better.

Alteration of these insecticides in the environment means that many unknown stable compounds could be formed. Some of these intermediates have chemical characteristics similar to the parent compounds and therefore can be detected by conventional methods such as gas-liquid chromatography with an electron-capture detection system. Other metabolites are not always detected by the same system, however. Since for the most part the toxicological properties of many of the stable intermediates, even if their identities are known, have not been fully studied, the implications of such contamination require much more attention than has been anticipated.

As shown in the following sections, the metabolic pathways of a compound in the environment could be multifold, and quite frequently several independent systems produce quite different stable end products from the same initial material. Discovery of TDE (DDD) formation in various ecosystems as a result of DDT application is a case in point, that is, it had been generally considered that DDE (generally in insects) and DDA (in insects and mammals), much less toxic analogs of DDT, were the only major metabolic products in general biological systems until microbial systems were examined. TDE itself is another potent insecticide with somewhat different insecticidal properties. This means that studies on the effects of TDE upon various ecosystems should be as important as those on DDT. Perhaps the above case is a very fortunate example since TDE is a well-known compound and its mass production has facilitated further toxicological evaluation of this particular terminal residue of DDT. In a recent year alone, for example, nine different stable metabolites have been reported to form from dieldrin, of which the chemical structures of many have only been tentatively proposed (see Fig. 10-3). Doubtlessly, many more stable intermediates will be discovered in the future and the toxicological implications elucidated.

10-3. DISTRIBUTION AND MOVEMENT OF INSECTICIDES IN THE ENVIRONMENT

By far the largest portion of applied insecticides, whether directed toward the atmosphere or toward foliage, eventually reaches the surface of soil in the form of insecticidal "fallout." Of course, soil applications require no movement at all.

A number of external factors play important roles in the eventual fate of such insecticidal deposits. The most important of them appear to be: (1) absorption to soil particles as well as organic matter in the soil; (2) leaching by water; (3) evaporation, including mechanical movement into the atmosphere by dust particles and water vapor; (4) metabolism by soil microorganisms; (5) physiochemical decomposition or alteration catalyzed by soil conditions such as pH or active clay crystals; (6) photodecomposition and (7) translocation through biological systems (e.g., food chains) to other ecosystems.

The most important factor determining persistence appears to be the chemical nature of the insecticide itself. Characteristics such as water solubility and polarity which in turn influence the lipophilic nature of the compound, as well as volatility and chemical reactivity-stability, are especially important. Thus through volatilization aldrin disappears much faster than dieldrin. Arsenicals, because of their insolubility in water and low vapor pressure, tend to accumulate in the topmost layer of soil.

With respect to the behavior of chlorinated hydrocarbons in soil or plants, their initial rate of disappearance follows the law of first-order kinetics. Thus by plotting the log amount of residue against time, linearly, a straight-line relationship is usually obtained.

In nature, however, this is usually a two-step reaction. First there occurs an initial fast phase, sometimes referred to as "dissipation," followed by a second slower phase, similarly referred to as "persistence" (*3,8*).

The most likely explanation for the above phenomena is that insecticides are quickly absorbed or translocated from the initial deposit site, thus escaping initial chemical degradation as well as "weathering" effects. Once this initial reaction is over, the insecticides are attacked by slower systems such as microbial degradation. Absorption in such reaction schemes is quite important. For instance, Lichtenstein and Schulz (*9*) showed that the amounts of aldrin and dieldrin recovered after treatment with aldrin were directly related to the amount of soil organic matter. The more organic matter present, the higher the amount of insecticide remaining in the soil. Also, as inferred previously, the more persistent insecticides are found in the top layer of soil (*10*) where most of the soil organic matter is located.

As far as persistent chlorinated hydrocarbon insecticides are concerned, absorption and translocation do not really decrease the total amount of insecticide present in the environment, though they secondarily affect the rate of degradation by influencing their availability to other reaction systems which may represent true degradation or chemical alteration systems.

The processes concerned with the chemical alteration of pesticides are (1) biological degradation (such as microbial degradation), (2) chemical and physiochemical reaction with soil components, and (3) photochemical degradation involving ultraviolet rays of the sun, for instance.

For the stable organic insecticides, unlike some of the unstable herbicides (*2*), chemical and physiochemical reactions do not appear to play an important role. In addition, photochemical processes appear to produce limited types of decomposition products, namely, rearrangements or decomposition reactions producing less strained molecules, at lower energy levels. In special cases photochemical reactions produce important end products such as photodieldrin, photoaldrin, and ketoendrin (*7*).

Biological degradation systems represent the most extensive processes of alteration of stable insecticides. In soil, particularly, microorganisms play an important role in altering the chemical nature of these insecticides. Also, the patterns of such microbial alteration are unpredictable and variable. For this reason the major emphasis of this chapter is on the microbial metabolism of insecticides. Other factors influencing the nature of insecticidal residues are discussed only briefly.

10-4. METABOLISM OF INSECTICIDES BY MICROORGANISMS

A. Chlorinated Hydrocarbon Insecticides

1. DDT and Analogs

By far the most extensively studied aspect of DDT metabolism by microorganisms is the process of direct dechlorination, which produces TDE (=DDD). This process was originally described by Kallman and Andrews (*11*) in 1963 utilizing a yeast. The formation of DDE, one of the major metabolic products in insects and other animals, was not observed. Barker and Morrison (*12*) found that TDE appeared in samples of DDT-fed mice that were kept for some time after sacrifice. Among several types of microorganisms isolated from the gut of a DDT-resistant female mouse, one isolate, *Proteus vulgaris*, was shown to actively convert DDT to TDE (*13*) and further to TDEE, 1,1′-bis(*p*-chlorophenyl)-2-chloroethylene and 1,1-bis(*p*-chlorophenyl)ethane (Fig. 10-1). This organism is known to be associated with some diseases of animals and is one of the primary invaders of animal tissue after death (*14*). TDE is the major DDT conversion product of microorganisms associated with digestive systems of various animals. Cultures of *Escherichia coli* and *Aerobacter aerogenes* isolated from rat feces produced large amounts of TDE in 24 hours (*15*); in addition, TDE was found in the feces and livers of rats given DDT by stomach tube but not in those of rats injected intraperitoneally with DDT. Bacteria isolated from the gut of stable flies converted DDT almost completely to TDE (90%) and to DDE (5%) under anaerobic conditions (*16*). Similar findings were made by Miskus et al. (*17*), who showed that 65% of DDT was converted to TDE in 24 hours when it was incubated

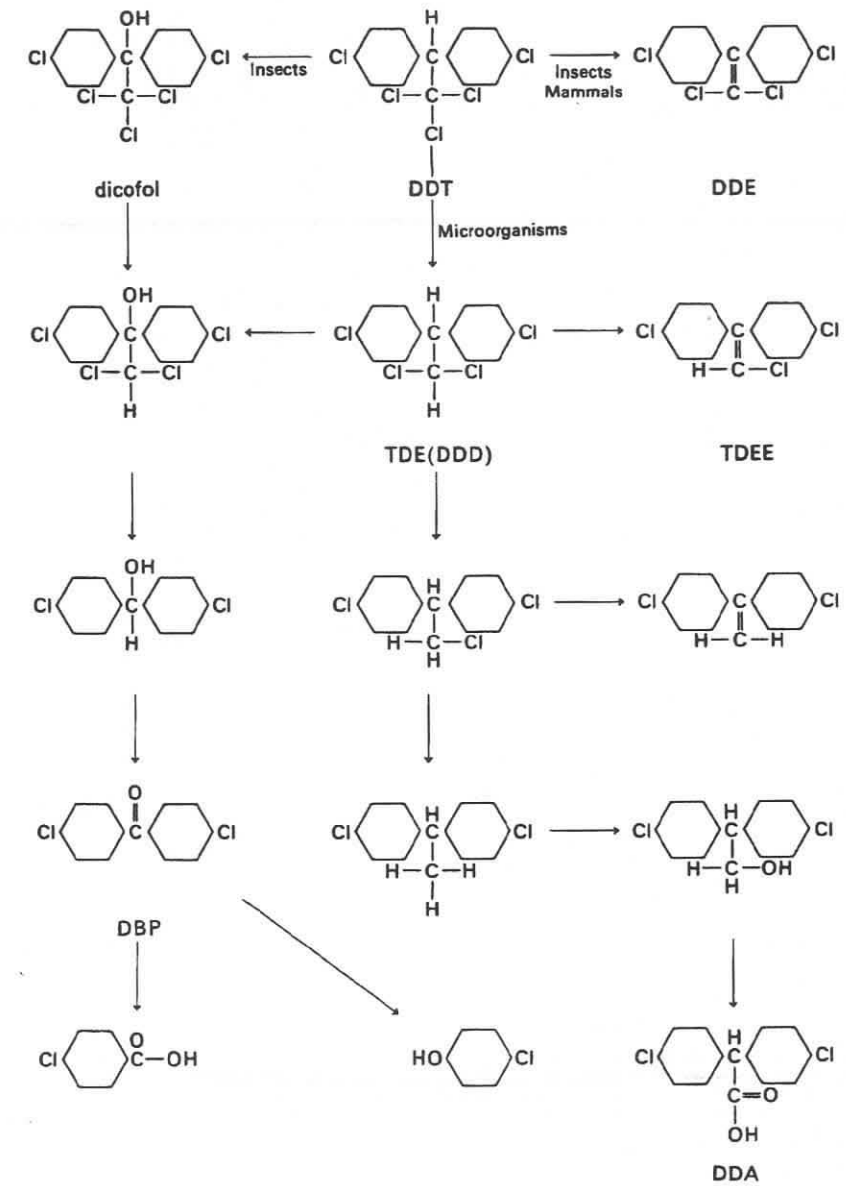

Fig. 10-1. Schematic diagram showing the general metabolic pattern of DDT by various biological systems. Not all intermediates have been detected and identified. The TDE-TDEE route appears to be the most common metabolic route for microorganisms.

with bovine rumen fluid, and by Braunberg and Beck (*18*) who expanded the previous work on rat intestinal microflora (*15*) to include many genera of common microorganisms for TDE production from DDT. Langlois (*19*), however, showed that *E. coli* grown in skim milk produced very little TDE from DDT in 7 days, whereas the same organism in a trypticase soy broth almost completed this conversion process in a similar period of time.

The ability to form TDE from DDT is not limited to microorganisms associated with animals. In fact, the initial discovery that TDE could be formed from DDT was made by Finley and Pillmore (*20*) with large numbers of water, soil, vegetation, and animal samples taken from an area where only DDT had been used.

Subsequent studies have revealed that the formation of TDE from DDT is not only a common reaction among various groups of soil microorganisms (*21*) and plant pathogenic bacteria (*22*) but also takes place in lake water and in aqueous solutions of reduced porphyrins (*17*).

It is apparent that this conversion process is the result of a direct reductive dechlorination which does not involve DDE as an intermediary metabolite as once speculated (*23*).

Although by far the most important microbial conversion product of DDT appears to be TDE (*24*), other metabolites are also formed in microbial systems. In many cases production of TDE was accompanied by DDE on a much smaller scale (*15*, *16*) and in some cases by additional minor metabolites such as DDA, dicofol (Kelthane), *p,p'*-dichlorobenzophenone (DBP), *p*-chlorobenzoic acid (BA), and *p,p'*-dichlorodiphenylmethane (DBM) (*21*). The majority of variants of the soil fungus *Trichoderma viride* that were tested produced TDE and a dicofol-like compound, while some variants exclusively produced DDA or DDE (*25*), thus indicating the presence of entirely different metabolic pathways or variations in relative activities of enzyme systems in metabolizing DDT among variants of the same species.

Studies on the metabolic fates of other DDT analogs by microorganisms are limited. Mendel et al. (*26*) found that 1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane is not produced from 1,1,1-trichloro-2,2-bis(*p*-ethylphenyl)ethane even after long incubation with *A. aerogenes*, the bacterium known to produce TDE from DDT. Methoxychlor was slowly degraded by the same organism into its olefinic derivative 1,1-bis(*p*-methoxyphenyl)-2,2-dichloroethylene and dechlorination product 1,1-bis(*p*-methoxyphenyl)-2,2-dichloroethane.

2. *Benzene Hexachloride*

Studies on the degradation of the γ isomer of BHC (or lindane) by microorganisms are again limited. Nevertheless, disappearance of BHC in the soil has long been observed (*27*,*28*). Bradbury (*29*) presented evidence that such loss in soils was attributable to slow bacterial decomposition. Subsequent

surveys showed that losses were associated with both microorganisms and alkalinity of the soil (*30*). γ-2,3,4,5,6-Pentachlorocyclohex-1-ene (γ-PCCH) was found in soils treated with BHC (*31*).

Recent work indicated that BHC is degraded by microorganisms that are present in submerged soil of Philippine rice fields. A second addition of BHC, 55 days after the initial application, caused quicker disappearance of BHC (*32*), implying that microevolution in the soil microorganisms had taken place, permitting the microflora to accommodate more BHC than they had previously.

Williams et al. (*33*) studied rumen bacteria and protozoan responses to BHC, in terms of gas formation, as the result of ulilization of this insecticide and found that *Isotricha intestinalis* and *Entodinium simplex* were active.

Allan (*34*) found that γ-BHC lost its effectiveness in simulated dipping liquid (cattle dip). Using the bacteria *Clostridium sporogenes* and *Bacillus coli*, which were found in such dips, they demonstrated that BHC gave rise to a trace of benzene and 1,2,3,5-tetrachlorobenzene. The γ isomer was utilized much faster than either the δ or α isomer.

3. *Chlorinated Cyclodiene Insecticides*

Cyclodiene insecticides include such important compounds as aldrin, dieldrin, heptachlor, isodrin, and endrin. These compounds also represent one of the most persistent group of insecticides. It has also been suggested that they generally have little effect upon bacterial and fungal growth (*35*).

Perhaps the best documented metabolic activity of soils involves the oxidation processes leading to the formulation of an epoxy ring (Fig. 10-2) from the unsaturated CH=CH bond of the unchlorinated (or less chlorinated) ring (*9*, *36–39*). Importance of soil microorganisms in the processes of epoxidation of cyclodiene insecticides has been suggested by some researchers from comparison studies on sterile and nonsterile soil samples (*9*). Evidence that microorganisms are able to epoxidize aldrin was provided by Korte et al. (*40*), who demonstrated that laboratory cultures of *Aspergillus niger*, *A. flavus*, *Penicillium notatum*, and *P. chrysogenum* converted aldrin-^{14}C to dieldrin and four other metabolites. Tu et al. (*41*) isolated a number of soil microorganisms from a farm soil which had been previously shown to convert aldrin to dieldrin. Among them the most active microorganisms were the various species of *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, *Nocardia*, *Streptomyces*, and *Micromonospora*. Three major types of fungi appeared to exist: (1) those that required about 3 weeks of adaptation period, after which the rate of dieldrin production suddenly increased, (2) those that demonstrated linear production of dieldrin from aldrin, and (3) those that produced moderately high amounts of dieldrin during the first 3 weeks of incubation but subsequently degraded the pesticide so that the level of dieldrin decreased thereafter.

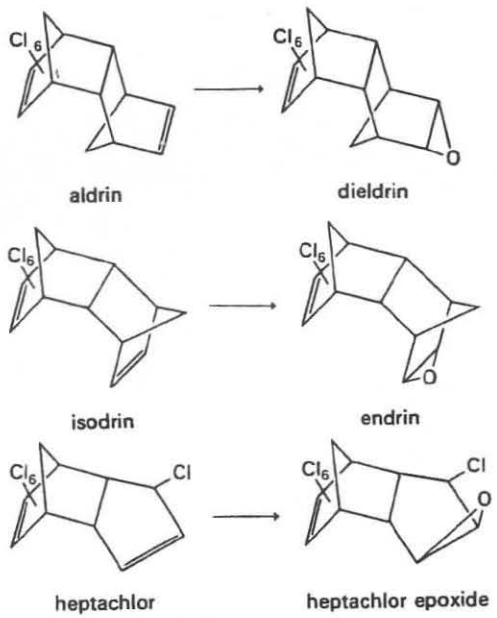

Fig. 10-2. Oxidation processes of cyclodiene compounds to yield more stable toxic intermediates. This is the system abundant in microorganisms.

While the expoxidation process is clearly defined as an "activation process" (production of more toxic metabolites than the parent compound), the significance of other metabolic activities of microorganisms are less clear. Following are examples of known metabolic activities of various microorganisms on cyclodiene insecticides.

a. Dieldrin. Dieldrin is probably the most stable insecticide among cyclodienes (Table 10-1). This probably is the reason why the role of microorganisms in the degradation of dieldrin was not demonstrated for many years. For instance, Chacko et al. (*24*) investigated the abilities of 17 species of fungi and actinomycetes, most of which degraded either PCNB or DDT or both, to degrade dieldrin. They found that none of them could metabolize this compound. Matsumura and Boush (*4*) studied 577 isolates from soil samples collected from places heavily contaminated with dieldrin and found that 10 of them degraded dieldrin to 3 to 10 unknown metabolites, including one that chromatographically matched 6,7-dihydroxydihydroaldrin. The microorganisms were *T. viride*, *Pseudomonas* species and *Bacillus* species. A subsequent study by Wedemeyer (*42*) with *A. aerogenes* showed that this microorganism exclusively produced a metabolite that matched 6,7-dihyoxydihydroaldrin in both gas and thin-layer chromatographic tests. A similar

TABLE 10-1

The Rate of Disappearance of Various Chlorinated Hydrocarbon Insecticides in Soil[a]

Chemicals	Time required for 95% disappearance, years	Average, years
Aldrin	1–6	3
Chlordane	3–5	4
DDT	4–30	10
Dieldrin	5–25	8
Heptachlor	3–5	3.5
Lindane	3–10	6.5
Telodrin	2–7	4

[a] From Edwards (*1*).

metabolic system was observed in *Pseudomonas melophthora*, a bacterial symbiote of the apple maggot (*43*). The work of Tu et al. (*41*) also indicated that a number of *Trichoderma*, *Fusarium*, and *Aspergillus* species, all isolated from aldrin-treated soils, were capable of degrading dieldrin.

The metabolic routes of dieldrin degradation in at least one instance proceed through a pathway entirely different (*44*) from mammalian systems (*7,45*). In the bacterial system two ketone derivatives of dieldrin appear to be the major metabolic products (Fig. 10-3) along with one alcohol, one aldehyde, and one acidic metabolite, all of which exist in small quantities. Since chromatographically identical spots for the above metabolites were also present in other microbial cultures (*4*), it is possible that such a metabolic route is a fairly common one among microorganisms.

b. Aldrin. Conversion of aldrin to dieldrin and subsequent degradation of aldrin through dieldrin metabolism have already been described in the foregoing section. Tu et al. (*41*) found that aldrin disappeared without the concurrent buildup of dieldrin in some of the microbial cultures and suggested direct degradation of aldrin. Research studies in our laboratory (*46*) also revealed that in some cultural media the metabolic products from aldrin were completely different from those from dieldrin. Korte et al. (*40*) also showed that a number of fungal isolates could degrade aldrin but not dieldrin.

c. Endrin, Heptachlor, and Chlordane Derivatives. Again, studies on this group of compounds are scarce. Poonawalla and Korte (*47*) found that metabolism of β-dihydroheptachlor-^{14}C by *A. niger* and *Penicillium urticae* proceeded very slowly: 3 and 4.5%, respectively, detected as more hydrophilic compounds in 15 days.

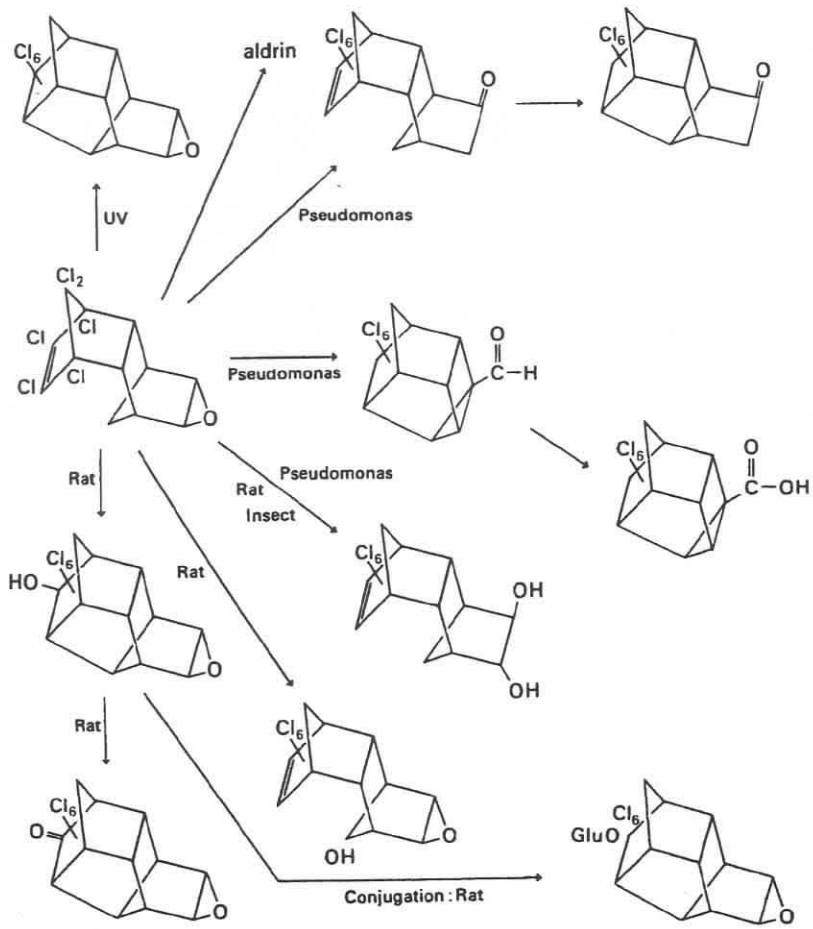

Fig. 10-3. Schematic diagram showing various routes of dieldrin metabolism. Some intermediates (e.g., **5**, **6**, **9**, and so on) are only tentatively identified (*44*,*45*).

Korte (*48*) reports that a culture of *A. flavus* metabolized endrin-^{14}C at rates of 2–3% (at 0.13 ppm) and 22% (at 0.022 ppm) into two compounds. The major metabolite was comparatively hydrophilic, and the minor metabolic product chromatographically (GLC) matched with ketoendrin (sometimes referred to as δ-keto-153). Williams et al. (*33*) found that rumen microorganisms, namely, *I. intestinalis* and *E. simplex*, particularly the former, utilized endosulfan (referred to as Thiodan) to produce carbon dioxide which was detected by a manometric method.

B. Organophosphorus and Carbamate Insecticides

These two groups, containing such well-known pesticides as parathion, methyl parathion, malathion, and carbaryl, are grouped here, not because of chemical similarity, but rather because they apparently have neither presented serious problems in soils as regards undesirable persistence nor demonstrated an extraordinary affinity for fat and resulting concentration within a food chain. Although considerable variation exists within each group, most of them are readily degraded in soil mainly by hydrolytic and oxidative means.

Studies on the degradation of insecticides by soil microorganisms are again limited. However, the fact that microorganisms are involved and actively participate in degrading insecticides in soil has long been suspected. Lichtenstein and Schulz (*49*) compared autoclaved and nonautoclaved wet and dry soils and concluded that the breakdown of parathion was brought about by the comparative numbers and metabolic activities of soil microorganisms. Ahmed and Casida (*50*) found that phorate and other dialkylphenyl phosphates and thiophosphates could be degraded in the laboratory by bacterial cultures of *Pseudomonas fluorescens* and *Thiobacillus thiooxidans*. Matsumura and Boush (*51*) showed that a soil fungus, *T. viride*, and a bacterium, *Pseudomonas* sp., could be involved in nature in degrading malathion. This view was derived from the fact that both cultures were originally isolated from a soil sample from an orchard previously treated with various pesticides. In a later paper they showed that this same fungal isolate had the ability to degrade diazinon, dichlorvos, parathion, and the carbamate, carbaryl (*25*). In addition, Boush and Matsumura (*43*) demonstrated that *Pseudomonas melophthora*, a bacterial symbiote of the apple maggot *Rhagoletis pomonella*, was capable of degrading diazinon, dichlorvos, parathion, and carbaryl through powerful esterases.

Several studies have indicated that the organophosphate insecticides at normal field dosage levels did not markedly alter the soil microflora. Sideropoulos et al. (*52*) noted that diazinon, malathion, phorate, and Systox, when added to field plots at 5–25 pounds/acre,* produced no stimulatory or inhibitory effects on fungal or *Streptomyces* counts. Diazinon at 10 pounds/acre actually resulted in an increase in total bacteria. While not involving soil microorganisms, Williams et al. (*33*) noted that concentrations of 500 ppm of Guthion, dimethoate, diazinon, or carbaryl did not inhibit or stimulate rumen bacteria. They also demonstrated diazinon uptake utilizing ^{14}C-labeled material with suspension of two rumen protozoa. More recently, Gunner and Zuckerman (*53*) showed the degradation of diazinon by an *Arthrobacter* sp. and a *Streptomyces* sp. and presented data suggesting a synergistic relationship between these two organisms in attacking the pyrimidinyl portion of the molecule.

* 1 pound/acre = 1.12 kg/ha.

Getzin and Rosefield (*54*) found that sterilization of soil samples by autoclaving destroyed approximately 90% of degradation activities of the soil sample against malathion, while a treatment by γ radiation (4 mrad, at a rate of 250,000 rad/hour) hardly reduced its metabolic activities. Subsequently, the above workers extracted a fraction with 0.2 *N* NaOH that actively degraded malathion, but not ciodrin, dichlorvos, or mevinphos. Since this factor was heat labile, it appeared to them to be an organic substance. In addition, the factor was stable in dilute acid (pH 2) and in 0.2 *N* NaOH, traits that do not appear to support a soil enzyme theory.

10-5. MICROBIAL-INSECTICIDAL RELATIONSHIPS IN SOIL

A. Effect upon Microbial Populations

Pesticides may alter the soil microflora population, both qualitatively and quantitatively, in several ways. The most obvious effect is that of direct toxicity of the applied insecticide to susceptible microbial species. Numerous studies, discussed in several excellent review papers (*55–57*), have appeared in recent years. Generally, they have attempted to correlate laboratory and field insecticide applications with reduction in microbial populations, especially beneficial microorganisms such as nitrogen-fixing bacteria. Although disturbances have been noted, especially at high levels in the laboratory, normal field application dosages have not been shown to create imbalances sufficient to result in significant reduction in soil fertility (*55*). The water solubility and vapor pressure of chlorinated hydrocarbon insecticides have also been considered to influence the toxicity of such compounds to fungi, the nearly insoluble cyclodienes (>1 ppm) being considerably less toxic than benzene hexachloride (>7 ppm), for example (*58*). Few studies have been concerned with microbial ecology. For example, should certain "antagonistic" species be affected, such as the common soil fungus *T. viride*, at least a temporary microbial fluctuation might be anticipated.

B. Ecological Considerations

It appears that the kind and number of soil microorganisms inhabiting a particular microniche are a function of complex interrelationships between the nutrient supply and environment. Or, expressed somewhat differently, each habitat has a definite "carrying capacity" limited by environment and nutrients, as far as microorganisms are concerned. Thus any changes in the habitat are reflected in qualitative and quantitative changes in the microbial population. As such, perhaps the microbial demand for assimilable carbon, the principal nutrient building block, would always exceed the supply (*59*) and the introduction of a new carbon source might contribute greatly to the

makeup of the microbial population. Also, the presence of a new carbon source might, through usual mutation and selection procedures, encourage the development of specific microorganisms capable of using the new introduced molecule as a carbon or energy source. It is also conceivable that insecticidal degradation could be brought about through the chemical action of microbial metabolic waste products or exoenzymes. Even more likely, the same mutation and selection factors might give rise to microorganisms possessing a detoxification mechanism. Since penicillinase development in certain previously penicillin-susceptible bacteria is observed, the possibility of an analogous situation in soil must be considered a possibility.

In fact there are two sets of data that suggest the occurrence of such evolutionary traits in nature: Raghu and MacRae (*32*) in laboratory studies found that a second addition of lindane to a soil sample, secured from a Philippine rice field, 55 days after the first addition of lindane to the same soil sample resulted in a faster disappearance of this chemical. Matsumura and Boush (*4*) screened over 500 soil isolates and found less than 12 bacteria and fungi capable of degrading dieldrin. All of the cultures were isolated from soil samples that had been heavily contaminated with dieldrin. At the same time, another possibility that the presence of insecticide-metabolizing microorganisms is purely coincidental does exist. Chacko et al. (*24*) surveyed laboratory collections of microorganisms and found that a number of them could degrade DDT; all of them were capable of degrading PCNB.

The above studies also suggest that the ease with which insecticide-degrading microorganisms are found is generally related to the overall stability of the pesticide.

With respect to microorganisms that degrade stable insecticides, such as dieldrin, it is increasingly becoming evident that few groups of microorganisms are involved. For instance, two different schools independently observed that the same species (*T. viride*) degrade aldrin and dieldrin (*4*, *41*). The result of metabolic tests of dieldrin on 16 laboratory variants of *T. viride* indicated that 4 variants were capable of degrading this compound despite that fact that they had not been subjected to dieldrin pressure (*25*). So far, members of only four genera, one fungal and three bacterial, are definitely known to be able to degrade dieldrin; they are *Trichoderma* (*4*, *41*), *Pseudomonas*, *Bacillus*, (*4*) and *Aerobacter* (*42*).

10-6. CONCLUSION

Although several workers, as previously mentioned, have demonstrated in the laboratory that some microorganisms are able to degrade even the most stable and persistent organic insecticides, no one has yet demonstrated

whether or not this occurs in nature, or even that these compounds serve as nutritional or energy sources for organisms. In fact there are no reports as yet that these chemicals have been shown to serve as sole nutritional carbon sources.

In view of such a paucity of information, it is difficult to formulate a sound theory with which to explain microbial degradation of these substances. The most crucial question is, why do microorganisms, in the presence of other natural nutrients, degrade insecticides often present in extremely minute quantities in their environments? Perhaps the answer lies in the unique nature of the pesticides, such as their lipophilic properties, for example, which permit them to be selectively absorbed in biological materials in relatively polar media such as soil and water (*60*) and allow their penetration of the normally selective cell wall. If this were the case, then it would be plausible to assume that the surviving organisms would be either tolerant, and capable of accumulating and storing the chemical in unaltered form, or able to degrade the foreign molecule (i.e., the insecticide) to a less toxic, perhaps assimilable form, or to a structure the organism could reject.

Certain aspects of the above argument are attractive since it is hard to accept the view that insecticides are primarily nutrition sources. Not only are the molecules stable and complex, and usually present in nature in minute quantities, but most microbial degradation studies, albeit in the laboratory, have been conducted under conditions involving excess nutrients. It is equally difficult to accept the view that "by chance" certain microorganisms possess enzymes only for the purpose of altering these complex molecules, though it is possible that in some cases broad-spectrum enzymes might be able to degrade such foreign compounds (*61–63*). It is also possible that since microorganisms are forced to pick up insecticides despite the presence of other nutrients in soil or cultural media the systems or strains that efficiently eliminate such "xenobiotics" may develop as the result of natural selection.

Acknowledging the incredible biological and chemical complexity of soil, we will probably find that many of these mechanisms, in addition to others undreamed of as yet, are important in understanding the fate of pesticides in our habitat.

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